

HEMATOXYLIN P.T.A. KIT

IVD *In vitro* diagnostic medical device

CE

Hematoxylin Phosphotungstic Acid kit for differentiating muscle tissues according to Mallory

INSTRUCTIONS FOR USE

REF Catalogue number: HPTA-100T (for 100 tests) HPTA-K-100 (4 x 100 mL)

Introduction

Hematoxylin P.T.A. kit is used for better visualization of elements of central nervous system, fibrins, but primarily for differentiation between smooth and striated muscle tissue.

Product description

- **HEMATOXYLIN P.T.A. KIT** – Kit for staining muscle tissue

The kit contains:	100 tests (HPTA-100T)	4 x 100 mL (HPTA-K-100)
Potassium permanganate, 0.5% solution	30 mL (KP05-OT-30)	100 mL (KP05-OT-100)
Sulfuric acid, 0.5% solution	30 mL (SK05-OT-30)	100 mL (SK05-OT-100)
Oxalic acid, 1% solution	30 mL (OKS1-OT-30)	100 mL (OKS1-OT-100)
Hematoxylin P.T.A.	100 mL (HPTA-OT-100)	100 mL (HPTA-OT-100)

Other sections and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue, BioWax Micro.
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparing the histological sections for staining

- Fixate the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μ m slices and place them on a VitroGnost glass slide.

NOTE:

Apply the reagent so it completely covers the section.

In order to avoid the section to get dry, we recommend using incubation chamber/plate.

Histological sections staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Apply 5 drops of Potassium permanganate, 0.5% solution and 5 drops of Sulfuric acid, 0.5% solution.	5 min
6.	Rinse in distilled (demi) water	
7.	Treat with Oxalic acid, 1% solution	5 min
8.	Rinse in distilled (demi) water	
9.	Dip the section into Hematoxylin P.T.A. Note: if you wish to accelerate the staining process, heat Hematoxylin P.T.A. for 20 seconds in microwave oven (500 W), remove the solution from the oven, immerse the section and incubate for 15 minutes. Macroscopically check for section coloration; in case of inadequate coloration, repeat the procedure until adequate coloration is achieved	incubate overnight at room temperature
10.	Rinse in distilled (demi) water	3-4 seconds
11.	Differentiate in 95% alcohol	several rapid dips
12.	Dehydrate using 100% alcohol (Histanol 100)	2 min
13.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with a VitroGnost cover glass.

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

Results

Dark blue - nuclei, fibrin, myofibril, astrocytoma, certain elastic fibers, myelin fibers, glial cells
Hues of brick red - collagen, cartilage, bone matrix

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in BioGnost's material safety data sheet.














Storing, stability and expiry date

Keep Hematoxylin P.T.A. kit in a tightly closed original package at temperature between 15°C and 25°C. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Culling, C.F.A. (1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
2. Lillie, R.D. (1945): Studies on selective staining of collagen with acid aniline dyes, J. Technical Methods, 25:1
3. Peers, J.H. (1941): A modification of Mallory's Phosphotungstic acid hematoxylin stain for formaldehyde-fixed tissue. *Arch. Pathol.* 32:446
4. Sheehan D.C. et Hrapchak, B.B. (1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.

HPTA-K-X, V4-EN3, 14 February 2017, AK/VR

 Refer to the supplied documentation	 Storage temperature range	 Number of tests in package	 Product code	 European Conformity
 Refer to supplied instructions	 Keep away from heat and sunlight	 Valid until	 Lot number	 Manufacturer
 For <i>in vitro</i> diagnostic use only	 Keep in dry place	 Caution - fragile		



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